

METABOLIZABLE ENERGY OF RATION ADDED WITH NANOCAPSULE OF TURMERIC EXTRACT ON BROILER CHICKEN

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ABSTRAK

Penelitian ini bertujuan untuk mengembangkan formulasi nanokapsul menggunakan ekstrak kunyit dan kitosan sebagai polimer serta sodium-tripolifosfat sebagai *cross linker*, untuk dipelajari kemampuannya dalam meningkatkan energi metabolis sejati ransum ayam broiler. Metode yang digunakan dalam formulasi nanokapsul adalah gelasi ionik. Metode untuk mengevaluasi energi metabolis sejati menggunakan total koleksi. Empat puluh delapan ekor ayam broiler dengan bobot badan rata-rata 900 g, dibagi secara acak ke dalam 8 perlakuan (satu perlakuan dipuasakan dan 7 perlakuan diberi ransum dengan penambahan *feed additive*), masing-masing enam ekor. Ayam dipuasakan sehari sebelum dan sesudah ayam diperlakukan dengan ransum, bobot dan kandungan nitrogen serta energy bruto pakan yang dikonsumsi serta ekskreta/ digesta ileum yang dikeluarkan diketahui untuk menghitung energi metabolis sejati. Telah ditemukan bahwa energy metabolis sejati ransum basal yang ditambah nanopartikel 0,1% (3370,66 kcal/kg) nyata lebih tinggi ($P<0,05$) dibanding ransum basal (3214,85 kcal/kg) dan ransum yang lainnya. Dapat disimpulkan bahwa formulasi nanokapsul menggunakan ekstrak kunyit dengan kitosan industri sebagai polimer dan sodium-tripolifosfat sebagai *cross-linker* berpotensi sebagai *feed additive* alternatif untuk meningkatkan energi metabolis sejati ransum pada ayam broiler.

Kata kunci: energi-metabolis-sejati, nanokapsul, ekstrak kunyit, kitosan, ayam broiler

ABSTRACT

This study was aimed to develop nanoparticle formulation using turmeric extract and industrial chitosan as the polymer and sodium-tripolyphosphate as cross linker, to evaluate its ability to improve true metabolizable energy on broiler chicken. Forty eight of broiler chickens with an average body weight of 900 g, were randomly divided into 8 treatments (one treatment was fasted and 7 were fed ration plus feed additive), containing six birds per treatment. The formulation of nanoparticle in this study used ionic gelation method. Method used to evaluate the metabolizable energy was total collection. Chickens were fasted one day before and after they were treated with rations. Nitrogen, gross energy and total weight of feed and excreta or ileal digesta released were analyzed to calculate the true metabolizable energy of the ration. It was found that the basal ration added with nanocapsule 0.1% had true metabolizable energy (3370.66 kcal/kg) significantly higher ($P<0.05$) than basal ration (3214.85 kcal/kg). The result of this study concluded that formulation of nanocapsule using turmeric extract and industrial chitosan as the polymer and sodium-tripolyphosphate as cross linker was potential to increase true metabolizable energy so it can be an alternative for broiler chicken feed additive.

Keywords: true-metabolizable-energy, nanocapsule, turmeric extract, chitosan, broiler chicken

INTRODUCTION

Antibiotics has facilitated the efficient production of poultry, allowing the consumer to purchase at a reasonable price on high quality of meat and eggs (Donoghue, 2003). A wide range of antibiotics are used in poultry not only to treat disease but also to maintain health, promote growth and enhance feed efficiency (Gaudin *et al.*, 2004). In particular, broiler chickens often grow rapidly with antibiotics to attain maximum weight within a short period of time (Nonga *et al.*, 2009). The uncontrolled and unlimited use of these antibiotics however may lead to the accumulation of undesirable residues in the treated animals and their products (Wachira *et al.*, 2011).

Controversy over the use of a fore mentioned antibiotics and high-energy rations call for an effort to find a feed additive from natural ingredients that have a potential to substitute the function of antibiotics to enhance feed efficiency included to improve metabolizable energy of the ration. One potential herbal medicine in Indonesia is curcumin which is the main active ingredient of turmeric rhizome. Several studies in vitro and in vivo have shown that turmeric can be as: antibacterial, anti-inflammatory, antitoxic, anti-hyperlipid, antioxidant and anticancer, but curcumin has low bioavailability (low solubility, poor absorption, fast pass, high rates of metabolism in the gut cells, rapid elimination) (Anand *et al.*, 2007). One reason for the low bioavailability of curcumin is water insoluble at acidic or neutral pH, and this causes absorption difficulty (Maiti *et al.*, 2007). This obstacle can be solved by the development of nanoparticle technology to improve drug delivery profile, especially for the less bioavailable chemical. Therefore, the application of curcumin needed polymers that are capable of carrying and being delivered to be absorbed properly, such as chitosan by nanotechnology (nanoencapsulation).

Chitosan is a natural biopolymer obtained by alkaline deacetylation of chitin /crab shell (Hejazi and Amiji, 2003). Chitosan has various excellent properties as biocompatible, bio-degradable, low toxic and not immunogenic. Chitosan is widely used together with TPP (sodium-Tripolyphosphate) polyanion in various nanoparticle formulation studies by ionic gelation method. Ionic gelation method is engaged to the forming of the complex by the two oppositely charged structures which then form nanoparticle

gel. Ionic gelation is a very simple and easy preparation method (Racovita *et al.*, 2009). This molecular weight takes influence in its solubility and viscosity. Short chain chitosan is dissolved easily in acidic organic solvents such as acetic acid, citric acid, and tartaric acid (Mao *et al.*, 2009).

This study was aimed to develop the nanoparticle formulation using turmeric extract with industrial chitosan as the polymer and sodium-tripolyphosphate as cross linker, to evaluate its ability as a feed additive for antibiotics substitute to enhance feed efficiency, specially to improve true metabolizable energy of ration in broiler chickens.

MATERIALS AND METHODS

The nanocapsule (840 ± 30.90 nm) was created by ionic gelation method using extracted turmeric:chitosan:TPP (sodium-tripolyphosphate) = 2%:2%:1% (w/v). Nanocapsule were produced from: (1). Turmeric extracted (20 g) dissolved in 100 mL ethanol 96% then added with chitosan (20 g) dissolved in 800 mL buffer acetic pH 4 by magnetic stirrer for 20 minutes, and then added with TPP (10 g) was dissolved in 100 mL aquades by magnetic stirrer for 20 minutes. Furthermore, the dispersed mixture was precipitated, filtered and oven-dried at 50°C and ground into powder to be mixed in the ration.

A total of 48 broiler chickens weighing \pm 900 g were placed in individual cages equipped with feed and water. Provision of feed additive has been done on 8 groups of chickens (1 group was fasted and 7 groups having feeding treatments as shown in Table 2) with 6 birds per group (Donkoh and Attah-Kotoku, 2009). A method for testing the metabolizable energy was by total collection method (modification from Revington *et al.*, 1991 and Adeola *et al.*, 1997). The 7th day of adaptation period was followed by the total collection. All chickens were fasted but drinking water was provided *ad libitum* on the first day of total collection. On the second day, 7 groups of chickens were fed basal rations (Table 1) plus feed additive (Table 2) and 1 group remained fasting to test endogenous N. On the third day, all chickens were fasted again. Excreta were collected by plastic bag from the second to the third day, completed 48 hours. On the fourth day, all chickens were slaughtered to take the ileal digesta.

Samples of feed and all of excreta or ileal

Table 1. Composition and Nutrient Contents of Basal Ration (BR)*

Feed Ingredients	Percentage
Milled yellow corn	52.00
Rice bran	12.50
Soybean meal	19.50
Fish meal	9.50
Palm oil	5.10
Limestone	0.30
NaCl salt	0.40
Top mix mineral-vitamin **	0.40
L-Lysine HCl	0.20
DL Methionine	0.10
TOTAL	100.00
Nutrient Contents	
Crude protein (%)	20.13
Metabolizable Energy (kcal/kg)	3201.77
Crude fat (%)	5.41
Crude fiber (%)	3.35
Calcium (%)	0.90
Available phosphorus (%)	0.43
Lysine (%)	1.29
Methionine (%)	0.5

* Standard nutrient requirements of broiler chickens 3-6 weeks (NRC, 1994): 20% protein; 1.0% Lys; 0.38% Met; energy 3200 kcal/kg, 0.9% Ca, 0.35% P_{av}

** Composition per kilogram of top mix mineral-vitamin : Ca 32.5%; P 10.0%; Fe 6.0 g; Mn 4 g; Iod 0.075 g; Zn 3.75 g; vit B₁₂ 0.5 mg; vit D₃ 50000 IU.

digesta were removed, weighed, dried and further ground to pass 1 mm sieve, then swabbed, included in labeled plastic bags and stored at 4°C for nitrogen and calorie analysis use. Gross energy was analyzed by bomb calorimeter (Gallenkamp auto bomb) and nitrogen contents of feed and excreta or ileal digesta was analyzed by AOAC (2006) to determine the effect of addition of nanocapsule feed additives on the true metabolizable energy of the ration. The AME

(Apparent Metabolizable Energy), AMEn (nitrogen corrected AME), TME (True Metabolizable Energy), and TMEn (nitrogen corrected TME) of the test were calculated as follows (Adeola *et al.*, 1997): $AME = (EI - EO) \div FI$; $AMEn = AME - (8.22 \times ANR \div FI)$; $TME = AME + (FEL \div FI)$; $TMEn = AMEn + (FEL \div FI) - (8.22 \times FNL \div FI)$, where EI is gross energy intake (kilocalories); EO is gross energy output in the excreta (kilocalories); FI is feed intake (grams); ANR is apparent nitrogen retention (grams) calculated as the difference between nitrogen intake and nitrogen output; FEL is the fasting energy loss from the group of the feed-deprived chickens (kilocalories); and FNL is fasting nitrogen loss from the group of feed-deprived chickens (grams).

The data metabolizable energy (AME, TME, AMEn and TMEn) was analyzed by analysis of variance and further significant differences used Least Significant Difference by SPSS-16 computer program .

RESULTS AND DISCUSSION

Metabolizable energy of rations added feed additive are shown in Table 2. The Value of TME of R1 that was based on research (Table 2) was equal to the value of R1 calculations based on the energy content of the feedstuff (Table 1 is 3202 kcal/kg). This shows that the method used to evaluate energy metabolizable is good. In granting BR plus NP 0.1% (R4) provided the greatest value of TME (3370.66) that differ significantly ($P < 0.05$) than the other rations included R1, R2, R3, R5, R6, R7 and R8, while R3 (BR plus turmeric extract 0.1%) gives the smallest value of ME. The administration R4 with 0.1% nanocapsule contained 0.04% turmeric extract, 0.04% chitosan and 0.02% STTP, produced TME that was greater value than the provision of R3 containing 0.1% turmeric extract. This provides evidence that the provision of nanocapsul was more effective to increase metabolizable energy ration. This is because in R4 nanochitosan is able to bind curcumin and take it, open the tigh-junction so that it can be absorbed better and get into the body cells, ionic bonding between curcumin and chitosan will be separated by a protease and lizozim that exist in the cell fluid. STTP has a negative charge that is able to bind chitosan that had a positive charge to form more robust capsule shell which can protect the chitosan from degradation by acid and protease in the stomach.

Table 2. Metabolizable Energy (AME, TME, AME_n and TME_n) of Ration Added *feed additive* on Broiler Chickens (kcal/kg)

Treatments	AME	TME	AME _n	TME _n
 (kcal/kg)			
R1, Basal Ration (BR)	3184.21 ^e ± 44.84	3214.85 ^e ± 44.84	3198.19 ^e ± 44.75	3228.05 ^e ± 44.75
R2, BR + Chitosan 0.1%	3021.23 ^c ± 76.72	3051.87 ^c ± 76.72	3036.40 ^c ± 76.66	3066.25 ^c ± 76.66
R3, BR + Turmeric E. 0.1%	2871.35 ^a ± 23.14	2901.99 ^a ± 23.14	2885.86 ^a ± 23.85	2915.72 ^a ± 23.85
R4, BR + Nanocapsule 0.1%	3340.02 ^f ± 44.90	3370.66 ^f ± 44.90	3352.02 ^f ± 44.94	3381.87 ^f ± 44.94
R5, BR + Nanocapsule 0.2%	3200.61 ^e ± 34.90	3231.24 ^e ± 34.90	3211.74 ^e ± 35.09	3241.59 ^e ± 35.09
R6, BR + Nanocapsule 0.3%	2964.11 ^b ± 21.80	2994.75 ^b ± 21.80	2976.59 ^b ± 21.43	3006.45 ^b ± 21.43
R7, BR + Nanocapsule 0.4%	2944.16 ^b ± 9.24	2974.79 ^b ± 9.24	2954.10 ^b ± 9.14	2983.96 ^b ± 9.14
R8, BR + Nanocapsule 0.5%	3119.14 ^d ± 37.69	3149.74 ^d ± 37.69	3135.79 ^d ± 37.62	3165.64 ^d ± 37.62

AME: apparent metabolizable energy, (AME_n) : nitrogen corrected AME; TME (True Metabolizable Energy), and TME_n (nitrogen corrected TME)

The average value in the same column with different superscripts indicate significant differences (P < 0.05).

Turmeric E. = Ethanol Extract of Turmeric, Nanocapsule is Nanocapsule of turmeric extracted by ethanol 96% and processed nanoencapsulation by ionic gelation method with chitosan as polymer and Sodium tripolyphosphate as cross linker.

While the turmeric extract /curcumin in intestines was no protect and unstable / degraded at neutral to alkaline pH. On the other hand, in the study of curcumin digestibility in R3 was 53.46% and R8 was 77.23%. Besides that, nanocurcumin, STTP, and nanochitosan have a function as antibiotics to reduce the population of pathogenic bacteria in gut.

The above items in accordance with the opinion of Sinaga *et al.* (2010) which stated that curcumin may affect the tone and contraction of the intestine, giving the low-dose will accelerate intestinal tone, but at high doses it will slow down and even stop the contractions of the small intestine. However, in the right dose, it will cause spontaneous contraction, so that the digestibility and absorption of food will increase. In mice and rabbits, curcumin can stimulate an increase in relaxation intestine resulting longer food in the small intestine and stimulates the secretion of hormones from the Brunner glands of the small intestine (Sinaga *et al.*, 2010). This is in line with an opinion that curcumin may increase the secretion of bile and pancreatic lipase activity, increased amylase, trypsin and chemotrypsin

(Chattopadhyay *et al.*, 2004). The same finding was reported by Rao *et al.* (2003) that curcumin increases spending stimulation of pancreatic enzymes and small intestine and decreased intestinal peristalsis, thereby providing nutrients absorption of products of digestion more. Pigs given 4 mg curcuminoids / kg body weight showed the highest protein digestibility, as giving the right dose curcuminoids can increase the digestibility of nutrients especially proteins, as curcuminoids to stimulate the secretion of hormones from the glands of Brunner in the intestinal wall, then this is the hormone which will stimulate increased secretion of digestive enzymes from the pancreas gland by enzymes in the small intestine enterokinase converted into enzyme trypsin which in turn can increase the digestibility of protein (Sinaga and Silalahi, 2012). Report of Dono (2012) showed that supplementation of turmeric powder 10 g / kg diet led the increasing of energy digestibility, the apparent metabolizable energy (AME), nitrogen corrected AME (AMEn), and total tract nitrogen retention. On the other hand, in the study of protein digestibility in R3 was 57.08% and R4

was 62.62%. Antimicrobial properties from curcumin, chitosan and STTP might help reducing the competition for available nutrients with the host, stimulate the growth of absorptive cells in the small-intestinal mucosa, and trigger the secretion of innate digestive enzymes. These enhancements should create a gut environment that is more suitable for optimal nutrient digestion and absorption, resulting in a greater nutrient and energy utilization efficiency.

The results of ME (Table 2) that are given basal ration plus chitosan 0.1% (R2) were smaller significantly ($P < 0.05$) than R1 because R2 had crude fat digestibility (43%) that was lower than others. Metabolizable energy derived from fat, protein and carbohydrates. Lipid is the largest contributor to energy. This is in line with an opinion stated that chitosan from shrimp shell can bind fats (FBC) averaging 416.5% for soybean oil, 503% for corn oil, and 400.8% for the sesame oil, and FBC value of original shrimp shell chitosan is higher than the original commercial FBC crab shell chitosan (Sigma Co.) (Sofia *et al.*, 2010). It is not as same as the opinion of Shi *et al.* (2005) that the optimum growth and feed conversion of broiler obtained by the addition of 0.5 to 1.0 g chitosan/kg feed, equivalent metabolizable energy yield from 0.05 to 0.1% in the diet has not changed. Ration that was added with nanocapsule 0.1% (R4) has a 63.46% fat digestibility. Most chitosan binds with curcumin and STTP from nanocapsule off, so there is little chitosan in the small intestine to bind dietary fat. There is also a free TPP that had alkaline pH, and will stimulate the secretion of lipase to digest fat. Both allow the energy digestibility in R4 was greater than the others, beside the increase of protein digestibility.

CONCLUSIONS

The nanocapsule of turmeric extract gave new hope as an alternative feed additive to replace antibiotic growth promoters. It was evident that the addition of 0.1% of nanocapsule in ration could improve true metabolizable energy of the ration.

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